

Claims

5        1. An insoluble protein or soluble or insoluble  
fragments thereof, said protein or said fragments being  
capable of binding tumor necrosis factor, and being a  
homogenous protein.

10       2. A homogenous protein selected from proteins  
containing the amino acid sequence of Figure 1, proteins  
containing fragments of said sequence, and proteins  
containing amino acid sequences analogous to the amino acid  
sequence of Figure 1, or to a fragment thereof, wherein said  
proteins are capable of binding tumor necrosis factor.

15       3. A protein of claim 2, wherein said protein has an  
approximate molecular weight of about 55 kilodaltons on a  
nonreducing SDS-polyacrylamide gel.

20       4. A homogenous protein selected from proteins  
containing the amino acid sequence of Figure 4, proteins  
containing fragments of said sequence, and proteins  
containing amino acid sequences analogous to the amino acid  
sequence of Figure 4, or to a fragment thereof, wherein said  
proteins are capable of binding tumor necrosis factor.

25       5. A protein of claim 4, wherein said protein has an  
approximate molecular weight of about 75 kilodaltons on a  
nonreducing SDS-polyacrylamide gel.

6. A protein of claim 2 which contains the amino acid sequence

5 Leu-Val-Pro-His-Leu-Gly-Asp-Arg-Glu-Lys-Arg-Asp-Ser-Val-  
-Cys-Pro-Gln-Gly-Lys-Tyr-Ile-His-Pro-Gln-X-Asn-Ser-Ile  
in which X stands for a non-determined amino acid residue.

10 7. A protein of claim 2 which contains the amino acid sequence

Ser-Thr-Pro-Glu-Lys-Glu-Gly-Glu-Leu-Glu-Gly-Thr-Thr-Thr-Lys.

15 8. A protein of claim 4 which contains the amino acid sequence

Leu-Pro-Ala-Gln-Val-Ala-Phe-X-Pro-Tyr-Ala-Pro-Glu-Pro-Gly-  
-Ser-Thr-Cys

in which X stands for a non-determined amino acid residue.

20 9. A protein of claim 4 which contains the amino acid sequence

Ile-X-Pro-Gly-Phe-Gly-Val-Ala-Tyr-Pro-Ala-Leu-Glu

in which X stands for a non-determined amino acid residue.

25 10. A protein of claim 4 which contains the amino acid sequence

Ser-Gln-Leu-Glu-Thr-Pro-Glu-Thr-Leu-Leu-Gly-Ser-Thr-Glu-  
-Glu-Lys-Pro-Leu.

30 11. A protein of claim 4 which contains the amino acid sequence Val-Phe-Cys-Thr.

12. A protein of claim 4 which contains the amino acid sequence

Asn-Gln-Pro-Gln-Ala-Pro-Gly-Val-Glu-Ala-Ser-Gly-Ala-Gly-  
-Glu-Ala.

35 13. A protein of claim 4 which contains the amino acid sequence Leu-Cys-Ala-Pro.

14. A protein of claim 4 which contains the amino acid sequence Val-Pro-His-Leu-Pro-Ala-Asp.

5 15. A protein of claim 4 which contains the amino acid sequence

Gly-Ser-Gln-Gly-Pro-Glu-Gln-Gln-X-X-Leu-Ile-X-Ala-Pro  
in which X stands for a non-determined amino acid residue.

10 16. DNA sequences encoding insoluble proteins or soluble or insoluble fragments thereof, which bind TNF, such DNA sequences being selected from the following:

15 (a) DNA sequences of Figure 1 or Figure 4 as well as their complementary strand, or those which include these sequences;

20 (b) DNA sequences which hybridize with sequences defined under (a) or fragments thereof;

25 (c) DNA sequences which, because of the degeneracy of the genetic code, do not hybridize with sequences of (a) and (b), but which code for polypeptides having exactly the same amino acid sequence.

30 17. DNA sequences which comprise a combination of two partial DNA sequences, the one partial sequence encoding soluble fragments of non-soluble proteins capable of binding TNF, and the other partial sequence encoding all domains except the first domain of the constant region of the heavy chain of human immunoglobulins such as IgG, IgA, IgM or IgE.

35 18. DNA sequences of claim 17, wherein said human immunoglobulins are IgM or IgG.

19. DNA sequences of claim 18, wherein said human IgG is of the IgG1 or IgG3 type.

20. A recombinant protein encoded by a DNA sequence of  
claim 16, or allelic variants thereof, or deletion,  
substitution, or addition analogues thereof.

21. A recombinant protein encoded by a DNA sequence of  
claim 17, or allelic variants thereof or deletion,  
substitution, or addition analogues thereof.

22. A recombinant protein encoded by a DNA sequence of  
claim 18, or allelic variants thereof or deletion,  
substitution, or addition analogues thereof.

23. A recombinant protein encoded by a DNA sequence of  
claim 19, or allelic variants thereof or deletion,  
substitution, or addition analogues thereof.

24. A vector containing a DNA sequence of claim 16 and  
which is suitable for expression of the protein encoded by  
said sequence in prokaryotic and eukaryotic host systems.

25. A prokaryotic or eukaryotic host system transformed  
with the vector of claim 24.

26. A host system of claim 25, wherein the host system  
is an insect or a mammalian cell.

27. A vector containing a DNA sequence of claim 17 and  
which is suitable for expression of the protein encoded by  
said sequence in prokaryotic and eukaryotic host systems.

28. A prokaryotic or eukaryotic host system transformed  
with the vector of claim 27.

29. A host system of claim 28, wherein the host system  
is an insect or a mammalian cell.

30. A vector containing a DNA sequence of claim 18 and  
which is suitable for expression of the protein encoded by  
5 said sequence in prokaryotic and eukaryotic host systems.

31. A prokaryotic or eukaryotic host system transformed  
with the vector of claim 30.

10 32. A host system of claim 31, wherein the host system  
is an insect or a mammalian cell.

15 33. A vector containing a DNA sequence of claim 19 and  
which is suitable for expression of the protein encoded by  
said sequence in prokaryotic and eukaryotic host systems.

34. A prokaryotic or eukaryotic host system transformed  
with the vector of claim 33.

20 35. A host system of claim 34, wherein the host system  
is an insect or a mammalian cell.

36. An antibody directed against a protein or fragments  
of a protein capable of binding tumor necrosis factor.

25 37. An antibody of claim 36 wherein said antibody is  
directed against a protein containing the amino acid sequence  
of Figure 1, fragments of said sequence, analogous sequences,  
or fragments of analogous sequences.

30 38. An antibody of claim 36 wherein said antibody is  
directed against a protein containing the amino acid sequence  
of Figure 4, fragments of said sequence, analogous sequences,  
or fragments of analogous sequences.

35 39. An antibody of claim 36 wherein said antibody is  
directed against a recombinant protein encoded by a DNA  
sequence of Figure 1 or Figure 4, complementary sequences,  
analogous sequences, or fragments of said sequences.

40. An antibody of claim 36 wherein said antibody is directed against a recombinant protein encoded by a DNA sequence which comprises a combination of two partial DNA sequences, one of which encodes soluble fragments of non-soluble proteins capable of binding TNF and the other of which encodes all domains except the first domain of the constant region of the heavy chain of human immunoglobulins such as IgG, IgA, IgM, or IgE.

41. An antibody of claim 40 wherein said antibody is directed against a recombinant protein wherein the human immunoglobulins are IgM or IgG.

42. An antibody of claim 40 wherein said antibody is directed against a recombinant protein wherein the IgG is of the IgG1 or IgG3 type.

43. A process for the isolation of an insoluble homogenous protein capable of binding tumor necrosis factor, which process comprises carrying out the following purification steps in the following sequence: production of a cell extract, immune affinity chromatography, and/or single or multiple ligand affinity chromatography, HPLC and SDS-PAGE, and, if desired, chemical or enzymatic cleavage of the isolated protein and/or conversion of said protein into a suitable salt.

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*add*  
*BB*  
*Crude*  
35 *Wes*  
*J*